CLAIMS

1. Process for the diagnosis of a human autoimmune disease, including pre-symptomatic diagnosis, said human autoimmune disease being associated with human endogenous retrovirus (HERV) having Superantigen (SAg) activity, comprising specifically detecting in a biological sample of human origin at least one of the following :

I- the mRNA of an expressed human endogenous retrovirus having Superantigen (SAg) activity, or fragments of such expressed retroviral mRNA, said retrovirus being associated with a given autoimmune disease, or

II- protein or peptide expressed by said retrovirus, or

III- antibodies specific to the proteins expressed by said endogenous retrovirus, or

IV- SAg activity specifically associated with said endogenous retrovirus,

detection of any of the species (I) to (IV) indicating presence of autoimmune disease or imminent onset of autoimmune disease.

2. Process according to claim 1 wherein the expressed retroviral mRNA is specifically detected by nucleic acid amplification using primers, one of which is specific for the poly(A) signals present in the 3' R-poly(A) sequences at the 3' extremity of the retrovirus.

3. Process according to claim 1 wherein the protein or peptide expressed by the endogenous retrovirus is detected using antibodies specific for the said retroviral protein or peptide.

4. Process according to claim 1 wherein the antibodies specific to retroviral protein are detected by use of the retroviral protein, or fragments thereof with which the antibodies specifically react.

5. Process according to claim 1 wherein SAg activity specifically associated with said HERV is detected, the biological sample being a biological fluid containing MHC Class II^+ cells or cells induced to express MHC Class II molecules, this sample being contacted with cells bearing one or more variable (V)-β T-cell receptor chains, and detecting preferential proliferation of the Vβ subset, or one of the vβ subsets characteristic of said autoimmune disease.

6. Process according to claim 1 wherein the autoimmune disease is type I diabetes and the associated retrovirus having SAg activity is $IDDMK_{1,2}$ 22 comprising the 5′ long terminal repeat shown in Figure 7A, the 3′ short terminal repeat shown in Figure 7B, or the env encoding sequences shown in Figure 7C, Figure 7D or Figure 7E, or variants thereof presenting approximately at least 90% sequence identity.

7. Process according to claim 6 wherein the expressed retroviral RNA is specifically detected by nucleic acid amplification using primers, one of which is specific for the poly(A) signals present in the 3' R-poly(A) sequences at the 3' extremity of IDDMK$_{1,2}$22.

8. Process according to claim 7 wherein the poly(A) specific primer is

5' TTTTTGAGTCCCCTTAGTATTTATT 3' or

5' T$_{(20)}$GAGTCCCCTTAGTATTTATT 3'

9. Process according to claim 6 wherein protein expressed by IDDMK$_{1,2}$22 is detected, said protein being either the protein encoded by the N-terminal moiety of the <u>env</u> coding region of IDDMK$_{1,2}$22 as illustrated in Figure 7D or 7G, or the protein encoded by the <u>pol</u> coding region, as illustrated in Figure 7H, or a protein having at least 90% homology with the illustrated protein, or a fragment of said proteins having at least 6 amino-acids.

10. Process according to claim 6 wherein antibodies specific for <u>env</u> or <u>pol</u> proteins expressed by IDDMK$_{1,2}$22 are detected using the <u>env</u> or <u>pol</u> proteins illustrated in Figure 7D, 7G or 7H, or a protein having at least 90% homology with the illustrated protein, or a fragment of said proteins having at least 6 amino-acids.

11. Human endogenous retrovirus having superantigen activity, and being associated with human autoimmune disease, said retrovirus being obtainable from RNA prepared from a biological sample originating from a human autoimmune source, by carrying out the following steps :

i) isolation of the 5′ R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3′ primer being complementary to known « primer binding sites » (pbs) and the 5′ primer being an oligonucleotide anchor ;

ii) isolation of the 3′ R-poly(A) ends corresponding to the 5′ R-U5 ends, by use of primers specific for the R regions isolated in step i) ;

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region ;

iv) amplification of the 5′ moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3′ end of the central pol region isolated in step iii) ;

v) amplification of the 3′ moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3′ R-poly(A) sequences isolated in step ii) ;

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

12. Proviral DNA of a retrovirus according to claim 11.

13. Proviral DNA according to claim 12 obtainable from a biological sample of human origin by :

i) obtaining retroviral RNA according to the method of claim 11, and further,

ii) generating a series of DNA probes from the retroviral RNA obtained in i);

iii) hybridising under stringent conditions, the probes on a genomic human DNA library ;

iv) isolation of the genomic sequences hybridising with the probes.

14. Nucleic acid molecule comprising fragments of the retroviral RNA or DNA according to any one of claims 11 to 13, said fragment having a length of at least 15 nucleotides and preferably at least 30 nucleotides.

15. Nucleic acid molecule according to claim 14, encoding SAg activity of the retrovirus.

16. Nucleic acid molecule according to claim 15 derived from an endogenous human retrovirus open reading frame and optionally containing at least one internal stop codon.

17. Nucleic acid molecule according to claim 15 or 16 comprising the retroviral env gene.

18. Nucleic acid molecule comprising a sequence complementary to the nucleic acid molecules of any one of claims 11 to 17.

19. Nucleic acid molecule according to claim 18 comprising a ribozyme or antisense molecule to a human retrovirus having SAg activity to a proviral DNA of said retrovirus or a fragment thereof.

20. Nucleic acid molecule capable of hybridizing in stringent conditions, with the nucleic acid molecules of any one of claims 11 to 19.

21. Vector comprising nucleic acid molecules of any one of claims 11 to 20.

22. Nucleic acid molecule comprising at least one of the sequences illustrated in Figures 7A, 7B, 7C, 7D, 7E, or a nucleic acid sequence encoding the POL protein shown in Figure 7H, or a sequence exhibiting at least 90% homology with any of these sequences, or a fragment of any of these sequences having at least 20 nucleotides, and preferably at least 40 nucleotides.

23. Nucleic acid molecule at least partially complementary to any of the sequences according to claim 22.

24. Nucleic acid molecule according to claim 22 comprising a ribozyme or antisense.

25. Nucleic acid molecule which is HERV $IDDMK_{1,2-22}$ comprising each of the sequences illustrated in Figures 7A, 7B, 7C, or sequences having at least 90% identity with these sequences, having a size of approximately 8.5 kb, having SAg activity encoded within the **env** region illustrated in Figure 7D or 7E, said SAg activity being specific for Vβ7 - TCR chains.

26. Protein or peptide having at least 6 amino acids, characterised in that :

- it exhibits SAg activity and optionally is capable of giving rise, directly or indirectly, to autoreactive T-cells targeting tissue characteristic of a given autoimmune disease ;
- it is encoded by a human endogenous retrovirus ;
- it is obtainable from biological samples of patients having autoimmune disease.

27. Protein or peptide according to claim 26, encoded by the env gene of the HERV, or a portion thereof.

28. Protein or peptide according to claim 27 corresponding to a protein or peptide resulting from a

premature translational stop, and/or from a frame shift in the translation of a retroviral open reading frame.

29. Protein or peptide according to any one of claims 26 to 28 obtainable by introducing viral DNA of claim 13 or fragments thereof, or corresponding synthetic DNA into a eukaryotic cell under conditions allowing the DNA to be expressed, and recovering said protein.

30. Protein according to any one of claims 26 to 29 comprising the amino acid sequence shown in Figure 7D, Figure 7F, Figure 7G, Figure 7H, or an amino acid sequence having at least 80% and preferably at least 90 % homology with the illustrated sequences, or a fragment of said sequence having at least 6 amino acids.

31. Antibodies capable of specifically recognising a protein or peptide according to any one of claims 26 to 30.

32. Antibodies according to claim 31 which are monoclonal.

33. Antibodies according to claim 31 or 32 which specifically recognise a HERV protein having SAg activity and which have the capacity to block SAg activity.

34. Cell-line transfected with and expressing a human retrovirus or a portion thereof or a nucleic acid molecule according to any one of claims 11 to 25.

35. Non-human cells transformed with and expressing a human retrovirus or a nucleic acid molecule according to any one of claims 11 to 25.

36. Cell-line or cells according to claim 34 or 35, said cell-lines or cells being MHC Class II^+ and expressing a protein having SAg activity.

37. Process for identifying substances capable of binding to retroviral protein or peptide according to any one of claims 26 to 30, comprising contacting the substance under test, optionally labelled with detectable marker, with the said retroviral protein or peptide having SAg activity, and detecting binding.

38. Process for identifying substances capable of blocking SAg activity of an endogenous retrovirus associated with autoimmune disease, comprising introducing the substance under test into an assay system comprising i) MHC Class II^+ cells functionally expressing retroviral protein or peptide according to any one of claims 26 to 30 and ii) cells bearing Vβ-T cell receptor chains of the family or families specifically stimulated by the HERV SAg expressed by the MHC Class II^+ cells, and determining the capacity

of the substance under test to diminish or block Vβ-specific stimulation by the retroviral SAg.

39. Process according to claim 38 wherein the cells bearing Vβ-T cell receptor chains are T-cell hybridoma and Vβ-specific stimulation is determined for example by measurement of IL-2 release, or measurement of T-cell proliferation.

40. Process according to claim 38 or 39, comprising an additional preliminary screening step for selecting substances capable of binding to retroviral protein having SAg activity, said screening step being according to claim 38.

41. Process for identifying substances capable of blocking transcription or translation of human endogenous retroviral (HERV) SAg-encoding nucleic acid sequences, said SAg being associated with a human autoimmune disease, comprising :

i) contacting the substance under test with cells expressing endogenous retroviral protein or peptide having SAg activity, according to any one of claims 26 to 30 and

ii) detecting loss of SAg protein expression using SAg protein markers such as specific, labelled anti-SAg antibodies.

42. Process according to claim 41 the cells expressing HERV protein having SAg activity are MHC

Class II$^+$ cells, and the process further comprises detection of loss of SAg activity by the process of claim 38.

43. Kit for screening substances capable of blocking SAg activity of a retrovirus associated with an autoimmune disease, or of blocking transcription or translation of the retroviral SAg protein, comprising :
- MHC Class II$^+$ cells transformed with and functionally expressing said retroviral SAg ;
- cells bearing Vβ T-cell receptor chains of the family or families specifically stimulated by the HERV SAg ;
- means to detect specific Vβ stimulation by HERV SAg ;
- optionally, labelled antibodies specifically binding to the retroviral SAg.

44. Protein or peptide derived from a retroviral SAg according to claim 26 wherein the protein is modified so as to be devoid of SAg activity and is capable of generating a immune response against SAg, involving either antibodies and/or T-cell responses.

45. Protein according to claim 44 wherein the modification consists of denaturation, or of a truncation, or of a deletion, insertion or replacement mutation of the SAg protein.

46. Protein according to claim 44 or 45 for use as a prophylactic or therapeutic vaccine against autoimmune disease associated with retroviral SAg.

47. Vaccine comprising an immunogenically effective amount of a protein according to claim 44 or 45 in association with a pharmaceutically acceptable carrier and optionally adjuvant.

48. Nucleic acid molecule encoding human retroviral SAg according to claim 15 or a modified form of said molecule for use as a prophylactic or therapeutic DNA vaccine against autoimmune disease associated with the retroviral SAg.

49. Substances identifiable by the process according to any one of claims 37 to 42 for use in therapy and/or prevention of autoimmune disease associated with the HERV SAg.

50. Use of substances capable of inhibiting retroviral function for the preparation of a medicament for use in therapy and/or prevention of autoimmune disease associated with retroviral SAg.

51. Use according to claim 50 wherein the substance capable of inhibiting retroviral function is Azido Deoxythymidine (A.Z.T.).

52. Use of substances capable of inhibiting retroviral SAg function for the preparation of a medicament for use in therapy of autoimmune disease associated with retroviral SAg.

53. Process for detecting human autoimmune disease associated with expression of human endogenous retrovirus Superantigen (SAg), said process comprising at least one of the following steps :

i) detecting the presence of any expressed retrovirus in a biological sample of human origin ;

ii) detecting the presence of SAg activity in a biological sample of human origin containing MHC Class II^+ cells.

54. Process according to claim 53 wherein the expressed retrovirus is detected by detection of reverse transcriptase activity.

55. Process according to claim 54 wherein the expressed retrovirus is detected by carrying out nucleic acid amplification reaction on RNA prepared from the biological sample, using as 3' primer a sequence complementary to known retroviral « primer binding sites » (pbs), and as 5' primer a non-specific anchor sequence.

56. Process according to claim 53 wherein the presence of SAg activity is detected by contacting the biological sample containing MHC Class II^+ cells with cells bearing one or more variable (V)-β T-cell receptor (TCR) chains and detecting preferential proliferation of a Vβ subset.

57. Process according to claim 56 wherein the cells bearing T-cell receptors are T-cell hybridoma bearing defined human Vβ domains.

58. Process for detecting SAg activity of an expressed human retrovirus associated with human autoimmune disease or of a portion of said retrovirus comprising :

i) transfecting expressed retroviral DNA or portions thereof into MHC Class II^+ antigen presenting cells under conditions in which the DNA is expressed,

ii) contacting the transfectants with cells bearing one or more defined (V)-β T-cell receptor chains, and

iii) determining whether the transfectant is capable of inducing preferential proliferation of a Vβ subset, the capacity to induce preferential proliferation being indicative of SAg activity within the transfected DNA or portion thereof.

59. Process for isolating and characterising a human retrovirus, particularly a human endogenous retrovirus (HERV), said retrovirus having SAg activity and being involved in human autoimmune disease, comprising the following steps :

i) isolation of the 5' R-U5 ends of expressed putative retroviral genomes using nucleic acid amplification, the 3' primer being complementary to known « primer binding sites » (pbs) ;

ii) isolation of the 3' R-poly(A) ends corresponding to the 5' R-U5 ends, by use of primers specific for the R regions isolated in step i) :

iii) amplification of the conserved RT-RNase H region within the pol gene by using degenerate primers corresponding to the conserved region ;

iv) amplification of the 5' moiety of the putative retroviral genome by using primers specific for the different U5 regions isolated in step i) in conjunction with a primer specific for the 3' end of the central pol region isolated in step iii) ;

v) amplification of the 3' moiety of the putative retroviral genome using primers specific for the central pol region isolated in step iii) in conjunction with primers specific for the poly(A) signals present in the 3' R-poly(A) sequences isolated in step ii) ;

vi) confirmation of the presence of an intact retroviral genome by amplification using primers specific for its predicted U5 and U3 regions.

60. Process according to claim 59 further comprising a step vii) of detecting SAg activity associated with the retrovirus, or portions thereof, said detection being carried out according to claim 58.

61. Transgenic animal including in its genome non-human cells according to claim 35.